Serial No. 10/530,240
Conf. No. 9656

Replacement Sheet
1/8

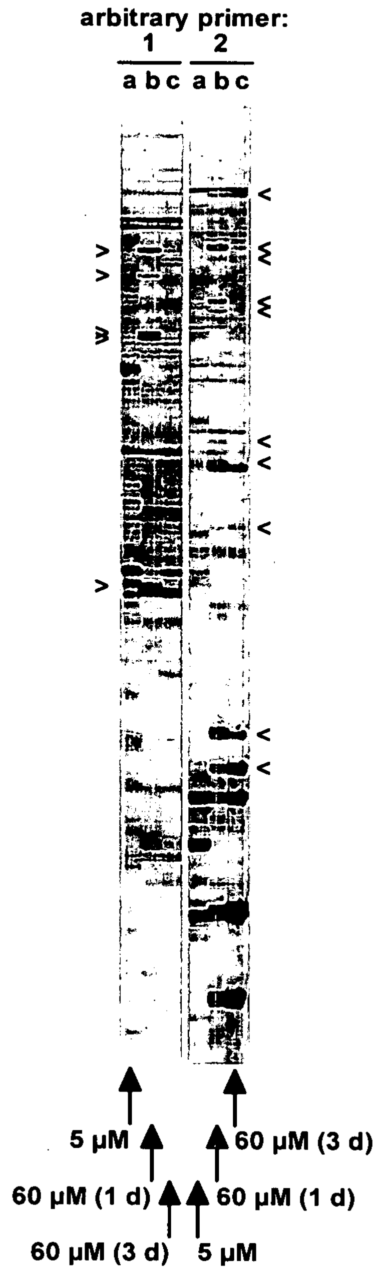

FIG. 1

```
>carbo#SD long
MSAILKRNVPIQRVGLPLTSYVSRWASALPTRTHPFYKLVDDSTTPVT
RSTLLSAHMVDTLLDENQQSRHENQHTDTSYKMYQGLKFVVKTLFTPS
KCHRHFSTSAHLSAMGRHQSPINIITSSTTKGPSLKPLKFSKSWDKPV
IGTVKDTGYYLKFAPESAAEKCTLHTYNGEYILDHFHYHWGKKDGEGA
EHFIDGKQYDIEFHFVHKKVGLTDPDARDAFAVLGVFGKADPRLKING
IWELLSPSTVLTVDSTRNVADVVPSKLLPSARDYFHYEGSLTTPTYGE
VVHWFVLNEPIAVPSEYLSALRQMQADKEGTVIDSNYRELQEVHNRPV
QRFKSDEQGRGEFDDISKNEDIVEDLSKLSGNFIRELVRKIYW
```

FIG. 2A

```
GAATTCGGCACGAGGGACAACTTTGCATAACTTTTACTGTCCATGTTTAACGTTTAGATCTAG   63
TACTAGTAGTCTACAAGAACAACTGTCAACAACTGTCAGATTATGTGTATAAACCAAGATGTC  126
                                                         M  S      2
TGCAATTCTTAAGAGAAACGTACCTATCCAAAGAGTCGGTCTCCCACTGACCTCCTATGTCAG  189
 A  I  L  K  R  N  V  P  I  Q  R  V  G  L  P  L  T  S  Y  V  S    23
TAGATGGGCTTCTGCTCTGCCCACCAGGACCCATCCTTTTTACAAGTTGGTTGATGACAGTAC  252
 R  W  A  S  A  L  P  T  R  T  H  P  F  Y  K  L  V  D  D  S  T    44
CACCCCAGTGACAAGGTCTACTCTTCTCAGTGCTCATATGGTTGACACCTTGCTAGATGAGAA  315
 T  P  V  T  R  S  T  L  L  S  A  H  M  V  D  T  L  L  D  E  N    65
CCAGCAGAGCAGACATGAAAACCAACACACAGACACGTCTTACAAAATGTACCAGGGATTAAA  378
 Q  Q  S  R  H  E  N  Q  H  T  D  T  S  Y  K  M  Y  Q  G  L  K    86
ATTTGTTGTAAAGACGCTGTTTACTCCATCGAAATGCCACCGTCACTTCTCCACATCAGCTCA  441
 F  V  V  K  T  L  F  T  P  S  K  C  H  R  H  F  S  T  S  A  H   107
TTTGTCTGCCATGGGTCGACATCAATCCCCCATCAATATAATCACCTCCAGTACGACCAAAGG  504
 L  S  A  M  G  R  H  Q  S  P  I  N  I  I  T  S  S  T  T  K  G   128
ACCGTCATTGAAACCGTTAAAATTTAGCAAGAGTTGGGACAAGCCAGTAATCGGCACCGTCAA  567
 P  S  L  K  P  L  K  F  S  K  S  W  D  K  P  V  I  G  T  V  K   149
AGATACTGGCTATTATCTTAAATTTGCACCAGAATCTGCAGCAGAGAAGTGCACATTGCATAC  630
 D  T  G  Y  Y  L  K  F  A  P  E  S  A  A  E  K  C  T  L  H  T   170
GTACAATGGTGAATATATCCTAGATCATTTCCATTATCACTGGGGGAAGAAGGATGGGGAAGG  693
 Y  N  G  E  Y  I  L  D  H  F  H  Y  H  W  G  K  K  D  G  E  G   191
AGCAGAGCATTTCATCGATGGAAAACAATACGACATCGAGTTCCACTTTGTACATAAAAAGGT  756
 A  E  H  F  I  D  G  K  Q  Y  D  I  E  F  H  F  V  H  K  K  V   212
TGGGTTGACTGATCCAGATGCTAGAGACGCTTTTGCTGTTTTGGGCGTTTTTGGAAAGGCCGA  819
 G  L  T  D  P  D  A  R  D  A  F  A  V  L  G  V  F  G  K  A  D   233
CCCTCGTTTGAAGATCAATGGAATCTGGGAGCTACTCTCACCGTCAACTGTCCTGACTGTCGA  882
 P  R  L  K  I  N  G  I  W  E  L  L  S  P  S  T  V  L  T  V  D   254
CTCAACACGAAACGTCGCTGATGTTGTTCCCTCTAAGCTTCTCCCAAGTGCCAGAGACTATTT  945
 S  T  R  N  V  A  D  V  V  P  S  K  L  L  P  S  A  R  D  Y  F   275
TCACTATGAAGGTTCTTTGACCACACCTACGTATGGTGAGGTTGTGCACTGGTTTGTTCTCAA 1008
 H  Y  E  G  S  L  T  T  P  T  Y  G  E  V  V  H  W  F  V  L  N   296
TGAACCCATAGCTGTCCCTAGTGAGTATCTGTCAGCTCTGAGACAGATGCAAGCTGACAAAGA 1071
 E  P  I  A  V  P  S  E  Y  L  S  A  L  R  Q  M  Q  A  D  K  E   317
AGGTACTGTGATTGACTCAAACTATCGAGAGCTTCAAGAAGTCCACAATCGACCTGTGCAACG 1134
 G  T  V  I  D  S  N  Y  R  E  L  Q  E  V  H  N  R  P  V  Q  R   338
ATTTAAGAGTGATGAGCAAGGGAGAGGAGAATTTGACGATATTTCTAAGAATGAGGACATTGT 1197
 F  K  S  D  E  Q  G  R  G  E  F  D  D  I  S  K  N  E  D  I  V   359
GGAGGACTTGTCTAAATTGTCTGGTAACTTTATTAGAGAGCTGGTCAGGAAGATATATTGGTG 1260
 E  D  L  S  K  L  S  G  N  F  I  R  E  L  V  R  K  I  Y  W      379
ACCTTTTTCTACACTTGTTAGAGTTTTAGGCCAGAATACATTTCATCATTTGGACTGTTATTT 1323
TGTGTACACTGCTTAGCAGTTTATATAAACACTACAATGCCATTATTATAATATAGCCAATGC 1386
TGTGATTTGA                                                        1396
```

FIG. 2B

```
SIA_SUBDO  MSAILKRNVPIQRVGLPLTSYVSRWASALPTRTHPFYKLVDDSTTPVTRSTLLSAHMVDTLLDENQQSRHENQHTDT  77
CAH2_HUMAN ------------------------------------------------------------------MSHH-------   4
           ~~~rec~~~~
SIA_SUBDO  SYKMYQGLKFVVKTLFTPSKCHRHESTSAHLSAMGRHQSPINIITSSTTKGPSLKPLKFSKSWDKPVIGTVKDTGYY 154
CAH2_HUMAN ---------WGYGKHNGPEHWHKDFPIAK-----GERQSPVDIDTHTAKYDPSLKPLSVSYDQATSLRILNNGHAFN  67
                    ╟ e-CAdom~~rec-s~~~      +++
SIA_SUBDO  LKFAPESAAEKCTLHTYNGEYILDHFHYHWGKKDGEGAEHFIDGKQYDIEFHFVHKKVGLTDP----DARDAFAVLG 227
CAH2_HUMAN VEFDDSQDKAVLKGGPLDGTYRLIQFHFHWGSLDGQGSEHTVDKKKYAAELHLVHWNTKYGDFGKAVQQPDGLAVLG 144
                                     Z Z        ■▲▲ ▲    ▲  ▲■Z■
SIA_SUBDO  VFGKADPRLKINGIWELLSPSTVLTVDSTRNVADVVPSKLLPSARDYFHYEGSLTTPTYGEVVHWFVLNEPIAVPSE 304
CAH2_HUMAN IFLKVG-SAKPGLQKVVDVLDSIKTKGKSADFTNFDPRGLLPESLDYWTYPGSLTTPPLLECVTWIVLKEPISVSSE 220
                                                            +    ++
SIA_SUBDO  YLSALRQMQADKEGTVIDSNYRELQEVHNRPVQRFKSDEQGRGEFDDISKNEDIVEDLSKLSGNFIRELVRKIYW   379
CAH2_HUMAN QVLKFRKLNFNGEG-----EPEELMVDNWRPAQPLK-NRQIKASFK-----------------------------   260
                                e-CAdom +╢                                      ~~~rec~~
```

FIG. 3A

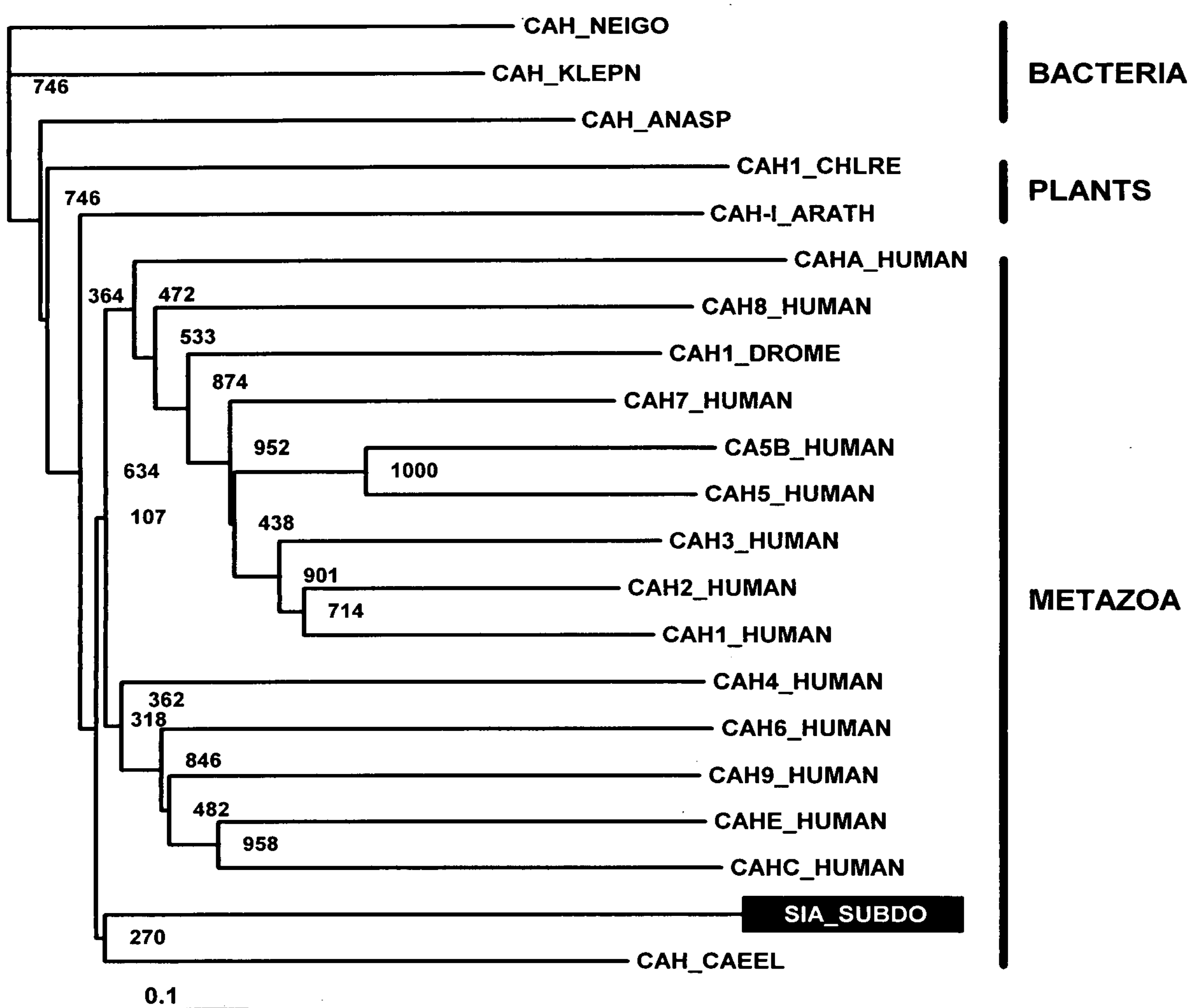
CAH_NEIGO
CAH_KLEPN
CAH_ANASP
CAH1_CHLRE
CAH-I_ARATH
CAHA_HUMAN
CAH8_HUMAN
CAH1_DROME
CAH7_HUMAN
CA5B_HUMAN
CAH5_HUMAN
CAH3_HUMAN
CAH2_HUMAN
CAH1_HUMAN
CAH4_HUMAN
CAH6_HUMAN
CAH9_HUMAN
CAHE_HUMAN
CAHC_HUMAN
SIA_SUBDO
CAH_CAEEL
746
746
364
472
533
874
952
1000
634
107
438
901
714
362
318
846
482
958
270
0.1
BACTERIA
PLANTS
METAZOA

FIG. 3B

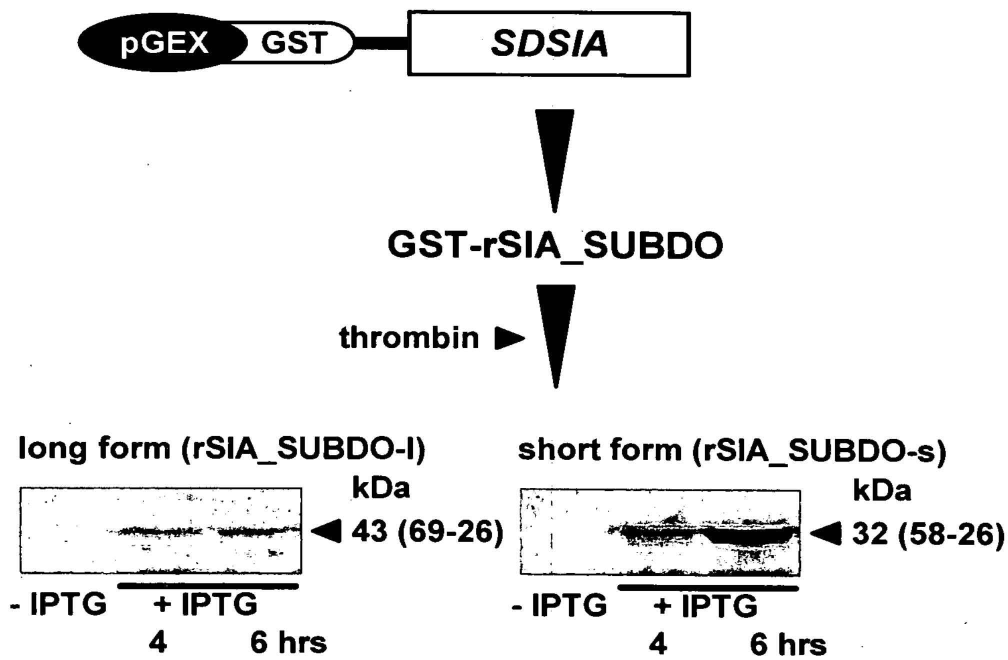
pGEX
GST
SDSIA
GST-rSIA_SUBDO
thrombin
long form (rSIA_SUBDO-l)
kDa
43 (69-26)
- IPTG
+ IPTG
4
6 hrs
short form (rSIA_SUBDO-s)
kDa
32 (58-26)
- IPTG
+ IPTG
4
6 hrs

FIG. 4

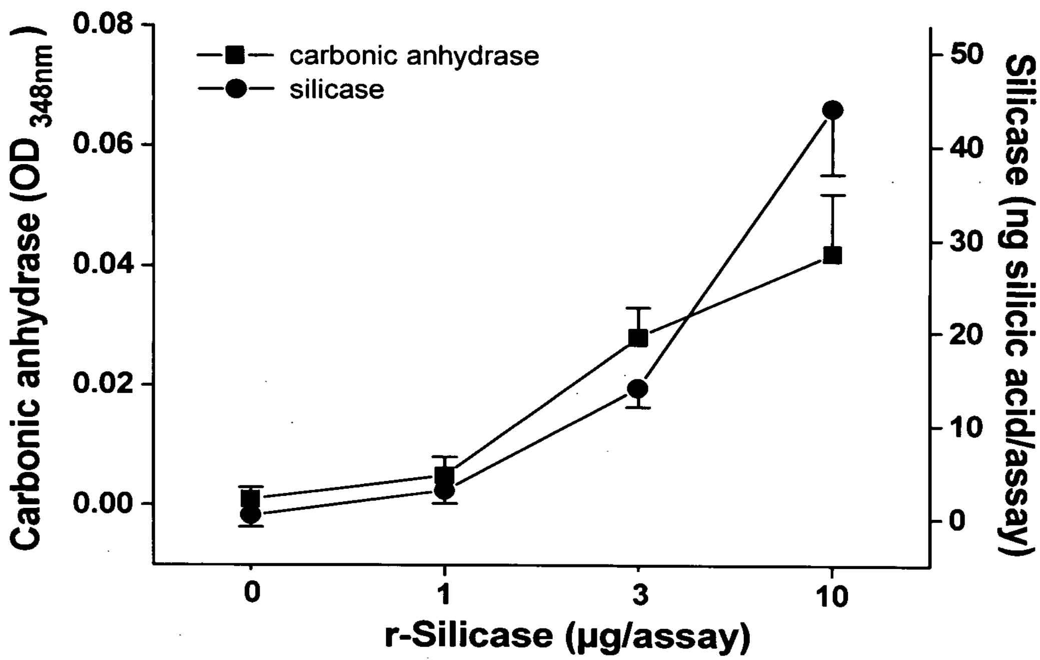
carbonic anhydrase
silicase
Carbonic anhydrase (OD 348nm)
0.08
0.06
0.04
0.02
0.00
Silicase (ng silicic acid/assay)
50
40
30
20
10
0
0
1
3
10
r-Silicase (µg/assay)

FIG. 5

FIG. 6A

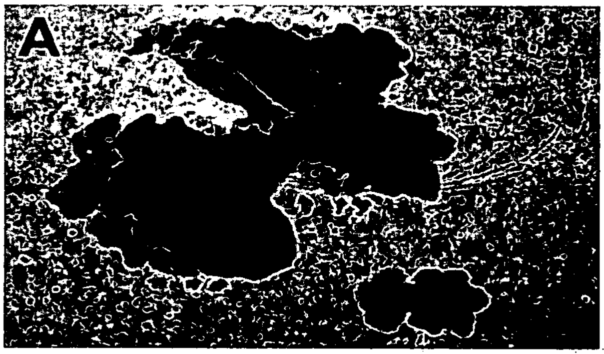

FIG. 6B

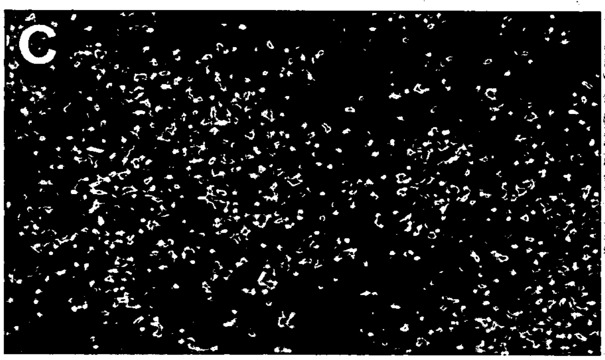

FIG. 6C

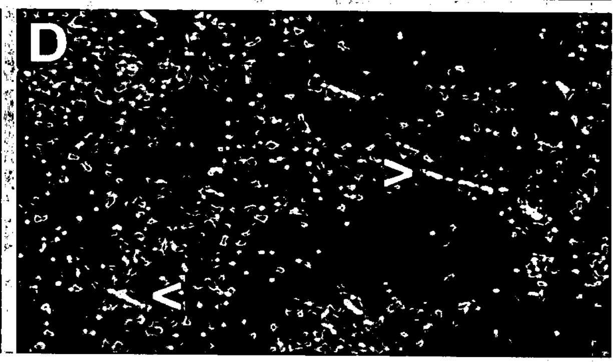

FIG. 6D

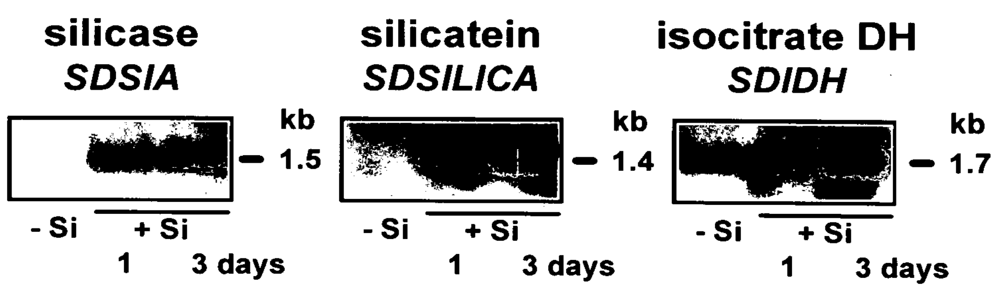

FIG. 7

[1] Reaction of silicase [hydration of CO_2]

$$CO_2 + H_2O \underset{}{\overset{\text{silicase}}{\rightleftharpoons}} H^+ + HCO_3^-$$

Effect on pH milieu
high metabolic activity

↓

oxidative respiration: CO_2 → release into the extracellular space

↓

$$CO_2 + H_2O \overset{\text{silicase}}{\rightleftharpoons} H^+ + HCO_3^-$$

↓ ↓

modulation of pH

FIG. 8A

[2] Reaction of silicase [ester splitting]

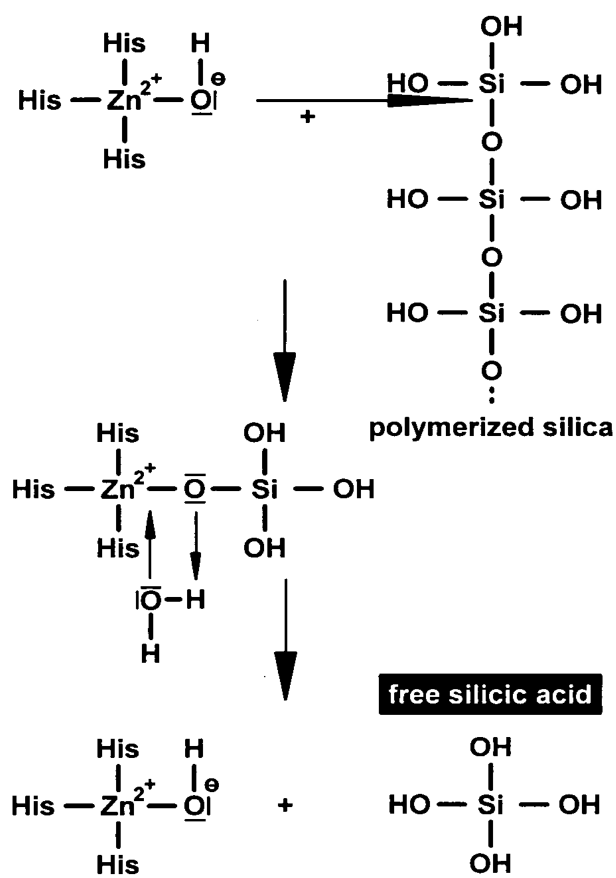

FIG. 8B

FIG. 9A

FIG. 9B